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## Evidence for a role of the epithelial glycoprotein 40 (Ep-CAM) in epithelial cell-cell adhesion.

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Recently we have demonstrated that a 40kD human epithelium-specific glycoprotein exhibit the features of a homophilic cell-cell adhesion molecule when expressed in transfected murine cells. We suggested the name Ep-CAM for this molecule (Litvinov et al., J. Cell Biol., 125: 437-446). Here we investigate the possible biological function of Ep-CAM in its natural environment--cells of epithelial origin. Immunolocalization of Ep-CAM in tissues and in cultures of epithelial/carcinoma cells showed that the majority of the Ep-CAM molecules are localized at cell-cell boundaries, predominantly along the whole lateral domain of polarized cells. In vitro, on single cells in suspension, the Ep-CAM molecules are present on the entire cell surface, and when the single cells grow attached, Ep-CAM is present at their pseudo-apical domain. During formation of intercellular contacts by such single cells, the majority of the Ep-CAM molecules are redistributed from the pseudoapical to the lateral domain of the cell membrane. Attachment of cells to the substrate does not cause redistribution of the molecules to the site of substrate attachment irrespective of the adhesive substrate (fibronectin, collagens, laminin, EHS-matrigel were tested). The monoclonal antibody 323/A3, reactive with the extracellular domain of the Ep-CAM molecule, has a strong negative effect on the aggregating behavior of COV362 ovarian carcinoma cells and RC-6 immortalized mammary epithelial cells. The mAb affected cell aggregation in both cell lines in the presence of Ca<sup>++</sup>, but with RC-6 cells the effect was more pronounced in low-calcium medium. The effects of the 323/A3 mAb on the already established intercellular contacts was not significant. The data presented demonstrate that the Ep-CAM molecules are functionally active in the epithelial and carcinoma cells tested, are capable of mediating Ca<sup>++</sup>-independent intercellular adhesions, and are not likely to be involved in cell-substrate adhesion.

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